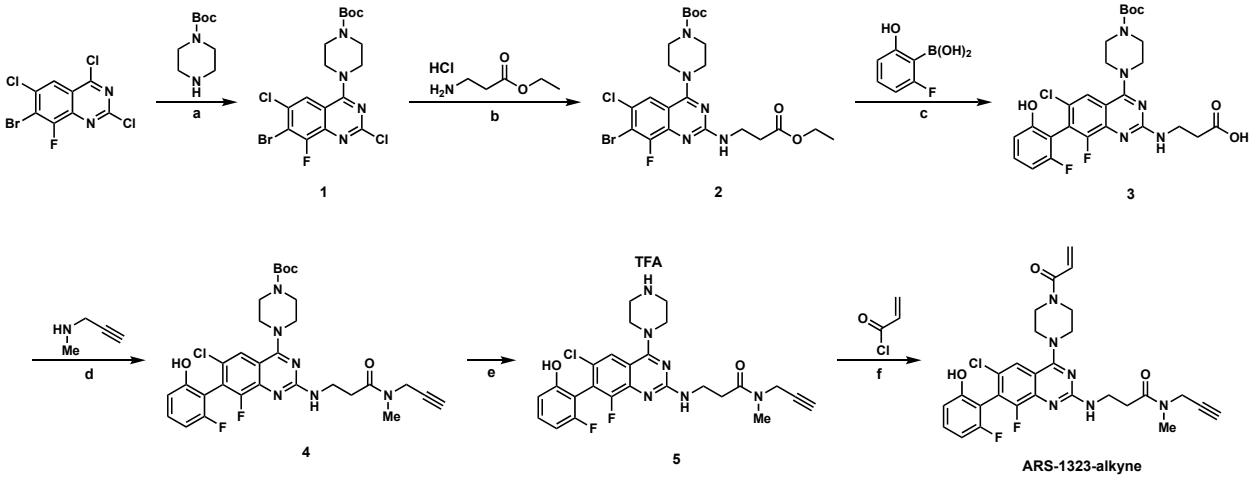
Supplemental Materials and Text

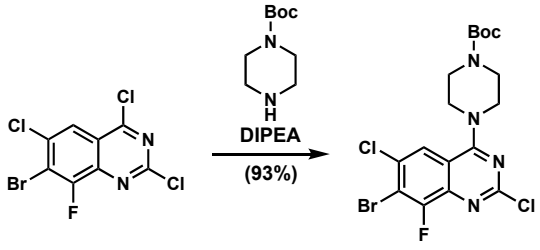
Text S1: Chemical synthesis details and methods

Nuclear magnetic resonance (NMR) spectra were recorded on a Varian spectrometer at 400 MHz or on a Bruker spectrometer at 500 MHz. Chemical shifts were reported as parts per million (ppm) from solvent references. Liquid chromatography-mass spectrometry (LC-MS) was performed on a Waters Xevo G2-XS QTof (0.6 mL/min) using an ACQUITY UPLC BEH C18 column (Waters) and a water/acetonitrile gradient (0.05 % formic acid) using Optima LC-MS grade solvents (Fisher Scientific). All other solvents were of ACS grade (Fisher Scientific, Millipore Sigma) and used without further purification. Commercially available reagents were used without further purification. Analytical thin-layer chromatography was performed with silica gel 60 F254 glass plates (Millipore Sigma). Silica gel chromatography was performed with RediSep Rf normal-phase silica flash columns using a CombiFlash Rf+ (Teledyne ISCO). Microwave reactions were performed using a Discover SP (CEM).



Reagents and conditions. (a) 1-Boc-piperazine, DIPEA, EtOH (93%); (b) β-alanine ethyl ester hydrochloride, DIPEA, iPrOH, 120 °C (63%); (c) 2-fluoro-6-hydroxyphenylboronic acid, Na_2CO_3,

$Pd(PPh_3)_4$, 1,4-dioxane, 90 °C, then $LiOH \cdot H_2O$, THF, EtOH, 60 °C (51%); (d) *N*-methylpropargylamine, DIPEA, HATU, MeCN, then DMAP, MeOH (96%); (e) TFA, CH_2Cl_2 (98%); (f) acryloyl chloride, DIPEA, CH_2Cl_2, 4 °C then $LiOH \cdot H_2O$, THF, H_2O (51%).



1

Compound 1. A reaction vessel was charged with 7-bromo-2,4,6-trichloro-8-fluoro-quinazoline (200 mg, 0.606 mmol) and 1-Boc-piperazine (113 mg, 0.605 mmol). Ethanol (3.0 mL) and *N*,*N*-diisopropylethylamine (211 µL, 1.21 mmol) were added and the reaction was allowed to stir at room temperature for 30 min. The mixture was partitioned between dichloromethane and water. The aqueous layer was extracted with dichloromethane (3X). The combined organics were washed with brine, dried over magnesium sulfate, filtered, and concentrated *in vacuo*. The crude was purified by flash chromatography over silica gel eluting with a gradient from 0% ethyl acetate-hexanes to 50% ethyl acetate-hexanes to afford compound 1 (271 mg, 0.564 mmol, 93%) as a white solid.

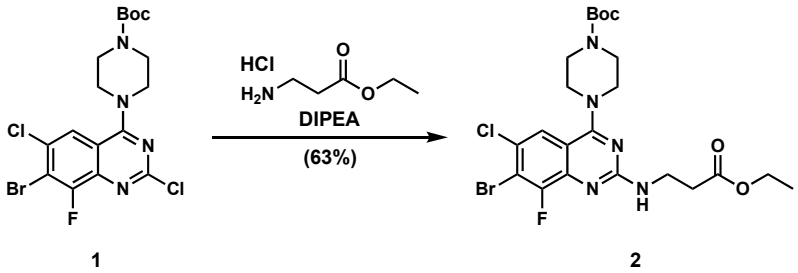
(Mixture of conformational isomers; major peaks reported)

^{1}H NMR (400 MHz, DMSO-d_6): δ 8.08 (d, *J* = 1.9 Hz, 1H), 3.92 (br, *J* = 6.1, 4.2 Hz, 4H), 3.55 (br, 4H), 1.43 (s, 9H).

^{13}C NMR (126 MHz, DMSO-d_6): δ 162.43 (d, *J* = 2.8 Hz), 156.56, 153.92 (d, *J* = 254.6 Hz), 153.86, 141.83 (d, *J* = 13.6 Hz), 129.08, 122.18 (d, *J* = 3.9 Hz), 114.65 (d, *J* = 3.0 Hz), 114.29 (d, *J* = 21.0 Hz), 79.28, 48.28, 41.85 (br), 28.07.

HRMS (ESI-TOF): *m/z*: calculated for $C_{17}H_{19}BrCl_2FN_4O_2$ $[M + H]^+$ 479.0047, found 479.0050

TLC: R_f = 0.8 (50% ethyl acetate-hexanes)



1 2

Compound 2. A microwave reaction vial was charged with compound 1 (200 mg, 0.417 mmol) and β-alanine ethyl ester hydrochloride (128 mg, 0.833 mmol). Isopropyl alcohol (2.6 mL) and *N*,*N*-diisopropylethylamine (145 µL, 0.742 mmol) were added and the reaction was heated in a microwave reactor at 120 °C for 3 h. The mixture was partitioned between dichloromethane and water. The aqueous layer was extracted with dichloromethane (3X). The combined organics were washed with brine, dried over magnesium sulfate, filtered, and concentrated *in vacuo*. The crude was purified by flash chromatography over silica gel eluting with a gradient from 0% ethyl acetate-hexanes to 50% ethyl acetate-hexanes to afford compound 2 (148 mg, 0.264 mmol, 63%) as an off-white solid.

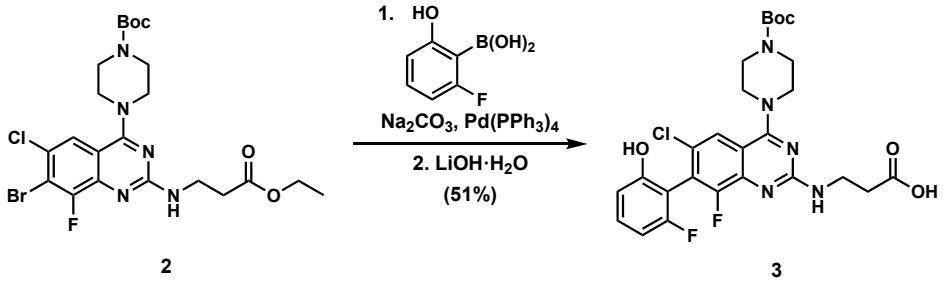
(Mixture of conformational isomers; major peaks reported)

^{1}H NMR (400 MHz, DMSO-d_6): δ 7.71 (s, 1H), 7.32 (br, 1H), 4.05 (q, *J* = 6.2, 5.7 Hz, 2H), 3.68 (br, 2H), 3.61 – 3.47 (m, 8H), 2.61 (br, 2H), 1.42 (s, 9H), 1.16 (t, *J* = 6.4 Hz, 3H).

^{13}C NMR (126 MHz, DMSO-d_6): δ 171.54, 163.59, 158.64, 153.95, 153.39 (d, *J* = 252.6 Hz), 143.21 (d, *J* = 11.5 Hz), 122.84, 121.29, 112.68 (d, *J* = 3.4 Hz), 112.50 (d, *J* = 19.9 Hz), 79.14, 59.90, 48.72, 42.27 (br), 36.92, 33.48, 28.06, 14.08.

HRMS (ESI-TOF) *m/z*: calculated for $C_{22}H_{29}BrClFN_5O_4$ $[M + H]^+$ 560.1070, found 560.1086

TLC: R_f = 0.7 (50% ethyl acetate-hexanes)



2 3

Compound 3. A reaction vessel was charged with compound 2 (50 mg, 0.0892 mmol), 2-fluoro-6-hydroxyphenylboronic acid (70 mg, 0.446 mmol), sodium carbonate (28 mg, 0.268 mmol), and tetrakis(triphenylphosphine)palladium(0) (10 mg, 0.0089 mmol). 1,4-Dioxane (1.2 mL) and water (0.315 mL) were added and the mixture was sparged with argon for 5 min. The mixture was then stirred at 90 °C under argon for 16 h. The mixture was cooled to room temperature before the addition to water (0.925 mL), methanol (1.2 mL), and lithium hydroxide monohydrate (37 mL, 0.892 mmol). The reaction was stirred at 60 °C for 30 min. The mixture was cooled to room temperature and partitioned between ethyl acetate and 5% citric acid. The aqueous layer was extracted with ethyl acetate (3X). The combined organics were washed with brine, dried over magnesium sulfate, filtered, and concentrated *in vacuo*. The crude was purified by flash chromatography over silica gel eluting with a gradient from 0% methanol-dichloromethane to 20% methanol-dichloromethane to afford compound 3 (26 mg, 0.0124 mmol, 51%) as an off-white solid.

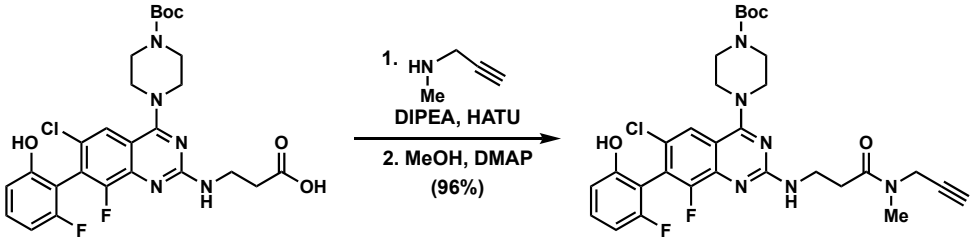
(Mixture of conformational isomers; major peaks reported)

^{1}H NMR (400 MHz, DMSO-d_6): δ 12.17 (br, 1H), 10.19 (br, 1H), 7.69 (d, J = 0.9 Hz, 1H), 7.36 – 7.28 (m, 1H), 7.17 (br, 1H), 6.83 (d, J = 8.3 Hz, 1H), 6.80 – 6.73 (m, 1H), 3.93 – 3.44 (m, 10H), 2.55 (br, 2H), 1.43 (s, 9H).

^{13}C NMR (126 MHz, DMSO-d_6): δ 173.28, 163.73, 160.98, 159.05, 158.47, 156.60 (d, *J* = 6.7 Hz), 153.99, 153.48 (d, *J* = 250.8 Hz), 142.90 (d, *J* = 5.8 Hz), 130.94 (d, *J* = 10.7 Hz), 122.81 (d, *J* = 322.5 Hz), 120.25, 113.40, 111.64, 107.88 (d, *J* = 18.9 Hz), 105.56 (d, *J* = 21.7 Hz), 79.15, 48.88, 42.39 (br), 37.07, 33.56, 28.09.

HRMS (ESI-TOF) *m/z*: calculated for $C_{26}H_{29}ClF_2N_5O_5$ $[M + H]^+$ 564.1820, found 564.1836

TLC: R_f = 0.6 (20% methanol-dichloromethane)



3 4

Compound 4. A reaction vessel was charged with compound 3 (42 mg, 0.0745 mmol). Acetonitrile (3.7 mL), *N*-methylpropargylamine (9.4 µL, 0.112 mmol), *N*,*N*-diisopropylethylamine (38.9 µL, 0.223 mmol), and HATU (42 mg, 0.112 mmol) were added and the reaction was stirred at room temperature for 30 min. The mixture was diluted with methanol (3.7 mL) and a catalytic amount of 4-dimethylaminopyridine was added before stirring the reaction at room temperature for 16 h. The mixture was concentrated *in vacuo* and then partitioned between ethyl acetate and water. The aqueous layer was extracted with ethyl acetate (3X). The combined organics were washed with brine, dried over magnesium sulfate, filtered, and concentrated *in vacuo*. The crude was purified by flash chromatography over silica gel eluting with a gradient from 0% methanol-dichloromethane to 10% methanol-dichloromethane to afford compound 4 (44 mg, 0.0715 mmol, 96%) as an off-white solid.

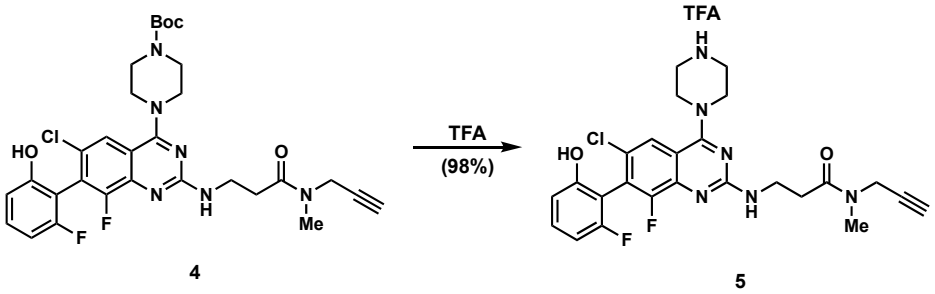
(Mixture of conformational isomers; major peaks reported)

^{1}H NMR (400 MHz, DMSO-d_6): δ 10.16 (s, 1H), 7.70 (s, 1H), 7.36 – 7.27 (m, 1H), 7.11 (br, 1H), 6.83 (d, *J* = 8.3 Hz, 1H), 6.80 – 6.73 (m, 1H), 4.13 (d, *J* = 2.3 Hz, 2H), 3.84 – 3.39 (m, 10H), 3.13 (t, *J* = 2.3 Hz, 1H), 3.03 (br, 3H), 2.66 (br, 2H), 1.43 (s, 9H).

^{13}C NMR (126 MHz, DMSO-d_6): δ 170.61, 163.80, 160.99, 159.05, 158.43, 156.58 (d, *J* = 6.6 Hz), 153.99, 153.55 (d, *J* = 253.7 Hz), 142.92 (d, *J* = 10.6 Hz), 131.19 – 130.65 (m), 122.83 (d, *J* = 321.5 Hz), 120.27, 113.41, 111.63, 107.88 (d, *J* = 18.9 Hz), 105.58 (d, *J* = 21.7 Hz), 79.75, 79.14, 73.99, 48.90, 42.39 (br), 37.16, 35.38, 34.11, 32.64, 28.08.

HRMS (ESI-TOF) *m/z*: calculated for $C_{30}H_{34}ClF_2N_6O_4$ $[M + H]^+$ 615.2293, found 615.2303

TLC: R_f = 0.3 (10% methanol-dichloromethane)

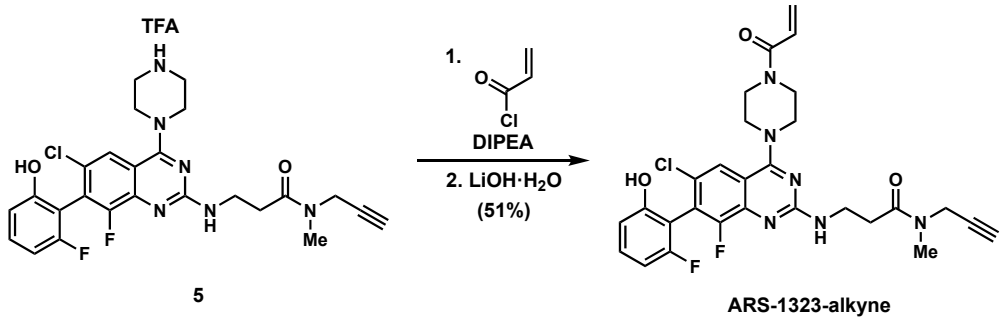


4 5

Compound 5. A reaction vessel was charged with compound 4 (44 mg, 0.0715 mmol). Dichloromethane (0.715 mL) and trifluoroacetic acid (0.715 mL) were added and the reaction was stirred at room temperature for 1 h. The mixture was concentrated *in vacuo* to afford compound 5 (44 mg, 0.0700 mmol, 98 %) as a yellow semisolid used in the next step without further purification.

HRMS (ESI-TOF) *m/z*: calculated for $C_{25}H_{26}ClF_2N_6O_2$ $[M + H]^+$ 515.1768, found 515.1799

TLC: R_f = 0.0 (10% methanol-dichloromethane)



ARS-1323-alkyne. A reaction vessel was charged with compound 5 (24 mg, 0.0382 mmol). Dichloromethane (1.9 mL) and *N*,*N*-diisopropylethylamine (13.3 µL, 0.0763 mmol) were added and the mixture was cooled to 4 °C before the addition of acryloyl chloride (3.4 µL, 0.0420 mmol). The reaction was stirred at 4 °C for 30 min. The mixture was concentrated *in vacuo* before the crude was resuspended in tetrahydrofuran (1.9 mL) and water (0.318 mL). Lithium hydroxide monohydrate (8 mg, 0.191 mmol) was added and the reaction was stirred at room temperature for 1 h. The mixture was partitioned between saturated ammonium chloride and ethyl acetate. The aqueous layer was extracted with ethyl acetate (3X). The combined organics were washed with brine, dried over magnesium sulfate, filtered, and concentrated *in vacuo*. The crude was purified by flash chromatography over silica gel eluting with a gradient from 0% methanol-ethyl acetate to 10% methanol-ethyl acetate to afford ARS-1323-alkyne (11 mg, 0.0193, 51 %) as an off-white solid.

(Mixture of conformational isomers, major peaks reported)

^{1}H NMR (400 MHz, DMSO-d_6): δ 10.16 (s, 1H), 7.73 (s, 1H), 7.38 – 7.26 (m, 1H), 7.14 (br, 1H), 6.92 – 6.69 (m, 3H), 6.16 (dd, *J* = 16.7, 2.3 Hz, 1H), 5.73 (dd, *J* = 10.4, 2.3 Hz, 1H), 4.13 (d, *J* = 2.4 Hz, 2H), 3.90 – 3.61 (m, 8H), 3.54 (br, 2H), 3.14 (t, *J* = 2.4 Hz, 1H), 3.03 (br, 3H), 2.67 (br, 2H).

^{13}C NMR (126 MHz, DMSO-d_6): δ 170.61, 164.45, 163.56, 160.98, 159.04, 158.41, 156.57 (d, *J* = 6.6 Hz), 153.54 (d, *J* = 249.1 Hz), 143.01, 130.96 (d, *J* = 10.9 Hz), 128.14, 127.70, 122.78 (d, *J* = 315.0 Hz), 120.32, 113.34, 111.63, 107.87 (d, *J* = 18.9 Hz), 105.59 (d, *J* = 21.8 Hz), 79.78, 74.02, 48.70, 44.44, 37.16, 35.38, 34.12, 32.64.

HRMS (ESI-TOF) *m/z*: calculated for $C_{28}H_{28}ClF_2N_6O_3$ $[M + H]^+$ 569.1874, found 569.1879

TLC: R_f = 0.5 (10% methanol-ethyl acetate)

Fig. S1. Genome-wide CRISPRi screening in the cancer cell line H358 identifies genes that influence cell growth and survival. (**A** and **B**) sgRNA phenotypes from a genome-wide CRISPRi screen in H358 grown in the presence of DMSO or ARS-1620. (**C** and **D**) Histograms of sgRNA phenotypes for each replicate of the screen among DMSO treated and ARS-1620 treated groups. Targeting and nontargeting sgRNAs included in the library are color coded black and grey respectively.

Fig. S2. Genome-wide CRISPRi screening in the cancer cell line MIA PaCa-2 identifies genes that influence cell growth and survival. (**A** and **B**) sgRNA phenotypes from a genome-wide CRISPRi screen in MIA PaCa-2 grown in the presence of DMSO or ARS-1620. (**C** and **D**) Histograms of sgRNA phenotypes for each replicate of the screen among DMSO treated and ARS-1620 treated groups. Targeting and nontargeting sgRNAs included in the library are color coded black and grey respectively.

Fig. S3. FOSL1 minimally modulates p-ERK and p-S6 phosphorylation dynamics. Immunoblots of H358 and MIA PaCa-2 cells transduced with a negative control sgRNA or sgRNAs targeting *FOSL1*. Cells were treated with DMSO or ARS-1620 (1 μM) for 24 hours. Cells were grown in 3D-spheroid culture. Immunoblots are representative of 2 biological replicates (same experiment as in Fig. 2E).

Fig. S4. Unique genetic dependencies within common signaling modules are revealed by evaluating ARS-1620 CRISPRi selection screens from distinct cancer cell lines. (**A**) Pathway map of CRISPRi screen gene phenotypes and Mann-Whitney *P* values from H358 ARS-1620 sensitization (red) and resistance (blue). Color intensities portray phenotype strength, and circle diameters represent $-\log_{10}$ Mann-Whitney *P* values derived from the CRISPRi screen. (**B**) Waterfall plot of expression data from the Cancer Cell Line Encyclopedia (CCLE) and phenotype magnitudes of 58 human RTKs from the CRISPRi screen in H358 cells. Z-score on the x-axis represents normalized mRNA expression data from RNA-seq, while color intensity and circle size represent phenotypes and Mann-Whitney *P* values from the CRISPRi screen respectively. Cells in (A) and (B) were grown in 2D-adherent culture. Phenotypes in (A) and (B) represent 2 biological replicates.

Fig. S5. Cellular response to $KRAS^{G12C}$ inhibition is mediated through phosphorylation changes in substrates of hit kinases. (**A**) H358 and (**B**) MIA PaCa-2 cells were grown in 3D-spheroid culture and treated with 3.3 μM ARS-1620 for 24 hours. All phosphopeptides are shown, with all significantly altered annotated substrates of EGFR (red) and CDK4 (purple) highlighted for H358, as well as significantly altered annotated substrates of FGFR (green) and CDK4 (purple) highlighted for MIA PaCa-2. Data represent 2 technical replicates of 3 biological replicates.

Fig. S6. CDK4/6i co-inhibition minimally alters p-AKT and p-ERK phosphorylation dynamics. Immunoblots of H358 (**A**) or MIA PaCa-2 (**B**) cells co-treated with a dose-range of ARS-1620 with DMSO or palbociclib (1 μM, CDK4/6i) for 24 h. Cells were grown in 3D-spheroid culture. Immunoblots are representative of 2 biological replicates (same experiment as in Fig. 6E).

Fig. S7. EGFRi co-treatment enhances the suppression of oncogenic Ras signaling by ARS-1620 in vivo. Immunoblot analysis of H358 xenograft tumors following 3 consecutive daily

doses of vehicle, ARS-1620 (100 mg/kg), erlotinib (100 mg/kg, EGFRi), or the combination 6 hours after last dose. Immunoblots represent 2 biological replicates.

Table S1. sgRNAs used for individual retesting. Table of the sequences, shown 5'-3', of the sgRNAs used to target the indicated genes. sgRNA1 and sgRNA2 refer to two distinct sgRNAs per target gene.

sgRNA	Protospacer sequence
CCND1 sgRNA1	GTGCGCCGACAGCCCTCTGG
CCND1 sgRNA2	GCAGCAGAGTCCGCACGCTC
CDK4 sgRNA1	GCATACACCCGAGCTCGGTC
CDK4 sgRNA2	GGCGGCCTGTGTCTATGGTC
CRKL sgRNA1	GTGGCTATTGGGAACAAGCT
CRKL sgRNA2	GCTGGGCAAAAGCACCCCGG
ELP3 sgRNA1	GATCTCTGGTGGCTCTGCTA
ELP3 sgRNA2	GCTACGGCGGCGCAGAAATG
ELP4 sgRNA1	GACTGGAGGCTCTAAGATGG
ELP4 sgRNA2	GGTAGTGTTGCCGCGAGTAC
FOSL1 sgRNA1	GAAGTCTCGGAACATGCCCG
FOSL1 sgRNA2	GGGCGGCTCTGCGGGGTACA
KAT6A sgRNA1	GGAGCTCACTGTCTCCAAGA
KAT6A sgRNA2	GCCTGGCGCAGAGCAGCGGG
KIF18A sgRNA1	GGGCGGACATTAAAGTGAAG
KIF18A sgRNA2	GTTGAGTCTGAAGCGCTGGG
MED24 sgRNA1	GCACCTAGAACTGGATTGTG
MED24 sgRNA2	GGATGCCTACCACCTAGAAC
TLN1 sgRNA1	GGGCGACCCGAGAAGCGGCG
TLN1 sgRNA2	GCCGAGGGCGACCCGAGAAG
TM2D1 sgRNA1	GCACAGGACACCAACGAGTC
TM2D1 sgRNA2	GCCGGAGCAGACGGACCAGA
TM2D2 sgRNA1	GCGGCCTCAACCACAACCCC
TM2D2 sgRNA2	GGCCTGACCACGCAGTTCTT
TM2D3 sgRNA1	GCAGCACGCGACACAAGGCG
TM2D3 sgRNA2	GCACGCGACACAAGGCGCGG
UNC50 sgRNA1	GGCCGGCTCCGTTGAGGGAA
UNC50 sgRNA2	GAGGGAAGCCCGCCCGGTGG

Data file S1. H358 ARS-1620 genome-wide CRISPRi sgRNA counts and phenotypes. Table of sgRNA read counts and phenotypes for the H358 CRISPRi DMSO and ARS-1620 screens. Illumina sequencing reads were processed as described in the methods to sgRNA read count data. The symbol Δ refers to the difference between ARS-1620 and DMSO data.

Data file S2. H358 ARS-1620 genome-wide CRISPRi gene phenotypes. Table of gene phenotypes and Mann-Whitney P-values for the H358 CRISPRi DMSO and ARS-1620 screens. The data was processed as described in the methods.

Data file S3. MIA PaCa-2 ARS-1620 genome-wide CRISPRi sgRNA counts and phenotypes. Table of sgRNA read counts and phenotypes for the MIA PaCa-2 CRISPRi DMSO and ARS-1620 screens. Illumina sequencing reads were processed as described in the methods to sgRNA read count data. The symbol Δ refers to the difference between ARS-1620 and DMSO data.

Data file S4. MIA PaCa-2 ARS-1620 genome-wide CRISPRi gene phenotypes. Table of gene phenotypes and Mann-Whitney P-values for the MIA PaCa-2 CRISPRi DMSO and ARS-1620 screens. The data was processed as described in the Methods.

Data file S5. ARS-1620 global phosphoproteomics. Table of phosphoproteomic results listed by protein name, the modified amino acid, the fold change in modification, and the calculated *P* value for each modified site. The data was processed as described in the Methods.